American Journal of Medical Genetics 68:86–90 (1997)

VACTERL With Hydrocephalus in Twins Due to Fanconi Anemia (FA): Mutation in the *FAC* Gene

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We present a dizygotic twin pair each with ventriculomegaly, a radial ray defect and multiple malformations in keeping with the VACTERL association. Molecular studies demonstrated that both are homozygous for IVS4 + 4 A → T, a mutation in the Fanconi anemia complementation group C gene. This is the first molecular proof that VACTERL with hydrocephalus may be the result of severe Fanconi anemia. Am. J. Med. Genet. 68: 86–90, 1997 © 1997 Wiley-Liss, Inc.

KEY WORDS: Fanconi anemia; VACTERL; hydrocephalus

INTRODUCTION

The association between vertebral defects, anal atresia, tracheo-esophageal fistula, renal malformation and radial ray defects (VATER) was first described by Quan and Smith [1973]. Subsequently, cardiovascular defects, other limb abnormalities and genital malformations [Temtamy and Miller, 1974; Apold et al., 1976; Evans et al., 1989] were added to the association (VACTERL). Later, hydrocephalus was recognised in a proportion of individuals with VACTERL association [Sujansky and Leonard, 1983; Briard et al., 1984; Aleksic et al., 1984; Hunter and MacMurray, 1987] (leading to the acronym VACTERL-H) and this combination has emerged as a distinct syndrome. Unlike VACTERL, which is usually sporadic, both autosomal recessive and X-linked recessive VACTERL-H pedigrees have been reported [Sujansky and Leonard, 1983; Briard et al., 1984; Hunter and MacMurray, 1987; Genuardi et al., 1993]. In addition, VACTERL-H appears to be associated with a worse outcome than VACTERL alone [Evans et al., 1989].

Fanconi anemia (FA) is an inherited form of aplastic anemia associated with increased spontaneous and mutagen-induced chromosome breakage and high risk of developing a malignancy. At least 90% of affected individuals have one or more congenital malformations including: radial ray defects (48%); genital, renal and urinary tract abnormalities (47%); and cardiac malformations (16%) [Auerbach et al., 1989]. Hydrocephalus is an uncommon, but recognised, association of FA affecting some 0.5–2.5% of patients [Alter, 1993; Kwee and Kuyt, 1989], and the overlap between VACTERL-H and FA has been the subject of several reports [Porteous et al., 1992; Toriello et al., 1993; Wang et al., 1993; Evans et al., 1994].

FA is a heterogeneous, autosomal recessive disorder and complementation studies indicate that at least 4 genes are responsible for this disease [Strathdee et al., 1992a]. The gene responsible for complementation group C (*FAC*) has been isolated and a number of mutations in this gene have been demonstrated [Strathdee et al., 1992b; Gibson et al., 1993; Verlander et al., 1994]. FA is relatively common in the Ashkenazi Jewish population, with an estimated carrier frequency of 1:100, and homozygosity for one particular mutation in *FAC,* a splice site mutation IVS4+4 A→T [Whitney et al., 1993], accounts for most cases of FA in this population [Whitney et al., 1994]. This mutation appears to be associated with a severe phenotype.

We report on a pair of dizygotic twins of Ashkenazi Jewish parents with VACTERL-H. Molecular genetic studies demonstrated that both are homozygous for the IVS4+4 A→T FA mutation.

CLINICAL REPORT

Following a prolonged period of infertility, a twin pregnancy was conceived by a South African Jewish couple of Ashkenazi descent, with the aid of follicle stimulating hormone (as urofollitropin; Metrodin®) treatment of the mother, who suffered from polycystic ovary disease. Maternal and paternal age was 28 and 30 years, respectively, and the family history was unremarkable. Antenatal ultrasound scans had detected dilatation of the cerebral ventricles and radial ray defects in both twins. Fetal blood sampling showed severe anemia of twin 1. The parents opted for termination of pregnancy which was performed at 18 weeks gestation.

Contract grant sponsor: Medical Research Council

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Received 28 August 1995; Accepted 5 February 1996

Autopsy Findings

Twin 1 was a male fetus of appropriate size for 18 weeks gestation (CRL 13.5 cm; foot length 2.5 cm; weight 128 g). External examination showed normal head circumference (OFC = 13.1 cm), a pointed chin, small "dysplastic" ears, radial deviation of the right hand and an absent right thumb; the left arm and hand was normal (Fig. 1). Internally there was a pericardial effusion, tracheo-esophageal fistula (TOF) of Gross type C and failure of lobation of the right lung. The duodenum was atretic, the intestine malrotated, and there was an ectopic right kidney sited in the midline at the pelvic brim; the left kidney was normally sited. The liver was pale and histology showed architectural disturbance with excess hemosiderin in hepatocytes and histiocytes on Perls' stain. Nuclear inclusions, suggestive of parvovirus B19 infection, were seen and immunohistochemistry confirmed the presence of Parvovirus antigen. The lateral ventricles of the brain were dilated, but the brain appeared otherwise normal. The aqueduct of Sylvius appeared narrow but preservation was suboptimal. Other organs were as expected apart from stress changes in the thymus and adrenals and apparently severe reduction in hemopoiesis in costal bone marrow. Roentgenograph showed hypoplasia of the right radius and absence of the radial ray in the right hand (Fig. 2); no vertebral abnormalities were identified.

Twin II was a female fetus also of appropriate size for 18 weeks gestation (CRL 13.2 cm; foot length 2.1 cm; weight 95 g) and without cranial enlargement (OFC 11.5 cm). Facial appearance was similar to that of the co-twin; there was radial deviation of the right hand but in this twin both thumbs were absent. Examination of the cardiovascular system demonstrated tetralogy of Fallot, a persistent left superior vena cava and a right-sided aortic arch. Lung lobation was abnormal; there was intestinal malrotation and an ectopic horseshoe kidney. The lateral cerebral ventricles were dilated but no other cerebral malformation could be identified; however, it was not possible to examine the aqueduct of Sylvius because of autolysis. A roentgenograph showed aplasia of the left radius and absence of the radial ray in the hands with no other skeletal abnormality (Fig. 2). The liver contained excess hemosiderin on histology and showed architectural disturbance, similar to that seen in twin 1, but no Parvovirus inclusions or antigen was detected.

Cytogenetic Analysis

Conventional G-banded chromosome analysis performed on lymphocytes from fetal blood samples

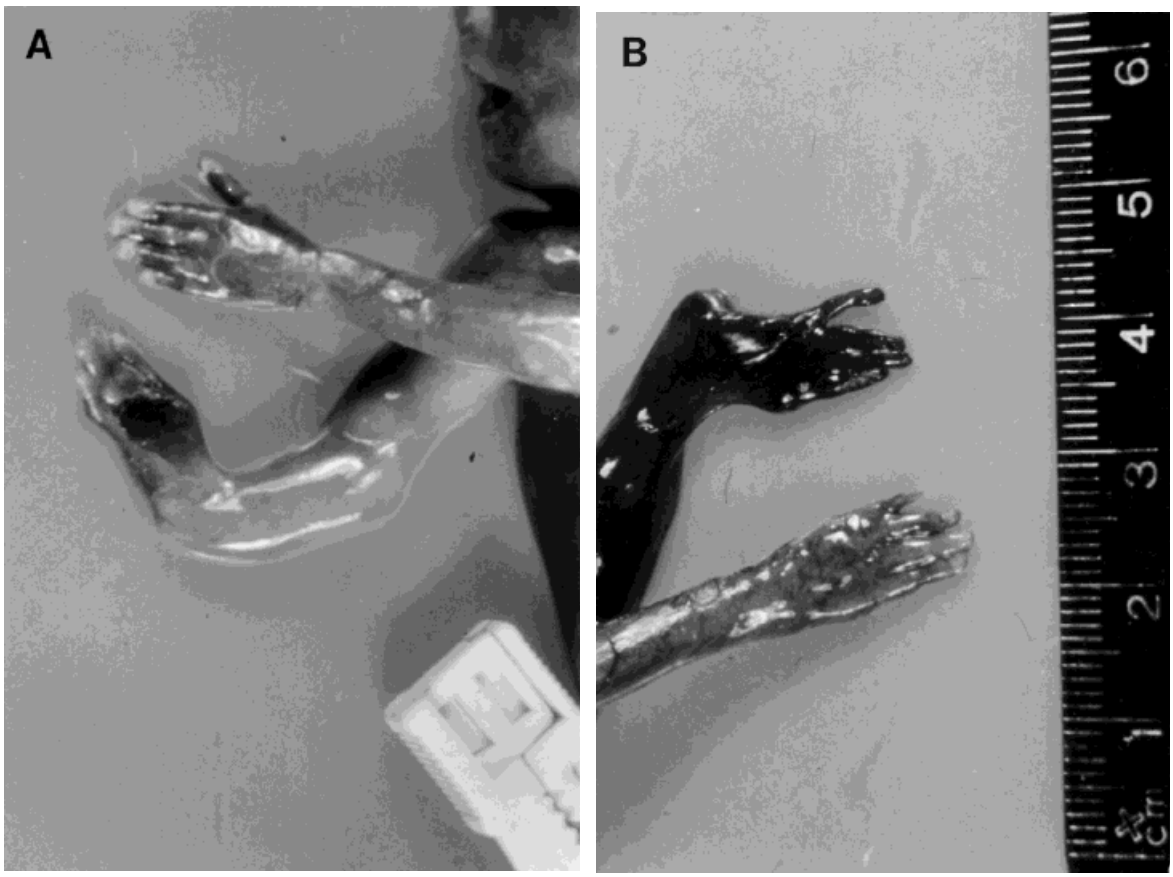

Fig. 1. **A,B:** Photographs of the hands of the twin fetuses showing absence of the right thumb in twin 1 and bilateral absence of thumbs in twin 2.

Fig. 2. Fetal postmortem roentgenograph. The right radius of twin 1 is hypoplastic and the radial ray is absent. The right radius of twin 2 is absent and the radial ray is absent in both hands.

demonstrated a normal karyotype, 46,XY (twin 1) and 46,XX (twin 2), in each fetus. There was no evidence of increased chromosome breakage on routine testing. Studies of hypersensitivity to DNA cross-linking agents, such as diepoxybutane (DEB), were not undertaken as the diagnosis of FA had not been entertained. Skin biopsies taken at autopsy for cytogenetic studies grew poorly and were not suitable for analysis.

Molecular Studies

In view of the clinical findings in the twins and the Ashkenazi Jewish ancestry of their parents, DNA extracted from the liver of twin I and the lung of twin II was tested for the common FA complementation group C (*FAC*) gene defect found in this racial group. Genomic DNA was extracted using the salt/chloroform method as described by Müllenbach et al. [1989]. Polymerase chain reaction (PCR) amplification was performed across the region of the IVS4+4 A→T mutation using primers F: −5′ CTCATATACTTTCAGCACTCAG 3′ and R: −5′ TTTCAAAAGTGATAAATATTAA***G**TAC 3′ which yield a product of 131 bp. The base ***G** generates an artificial *Sca1* restriction site in the normal *FAC* gene which is absent in alleles with the IVS4+4 A→T mutation. Digestion of the product with *Sca1* yields fragments of 108 and 23 bp for normal alleles. PCR conditions were 94°C for 1 minute; 50°C for 1 minute; and 72°C for 1 minute with a final extension step of 72°C for 5 minutes. Figure 3A shows that in both twins the PCR product of 131 bp is undigested by *Sca1,* indicating homozygosity for the IVS4+4 A→T FA mutation. Testing of DNA from parental blood samples generates bands of 131 and 108 bp showing that both parents possess one normal allele and one carrying IVS4+4 A→T. The mutation was confirmed by DNA sequencing (Fig. 3B).

DISCUSSION

In the cases described in this report it has been possible to show, for the first time, that not only can apparent cases of VACTERL-H show increased spontaneous and inducible chromosome breakage, but also that they may be due to mutation of a known FA gene. This is obviously of importance to the family described, since they may be offered prenatal molecular diagnosis in future pregnancies. It is also a further step towards understanding the pathogenesis of the VACTERL-H syndrome and the VACTERL association.

Most cases of the VACTERL association are sporadic and the recurrence risk is considered low. In contrast, VACTERL-H, which for some time has been thought to be a separate condition, has been reported in multiple members of several families, suggesting a single gene defect may be responsible and autosomal recessive [Sujansky and Leonard, 1983; Briard et al., 1984; Corsello and Guiffrè, 1994] and X-linked patterns of inheritance have been described [Hunter and MacMurray, 1987; Genuardi et al., 1993; Wang et al., 1993]. The prognosis for survival and mental handicap in VACTERL-H appears much poorer than the prognosis for VACTERL alone [Evans et al., 1989]: of 16 infants reviewed by Briard et al. [1984], 13 were stillborn or died in early neonatal life. However, this is not a constant finding since 2 of 3 cases reported by Iafolla et al. [1991] had a good outcome following neurosurgery. The twins reported here show considerable similarity to the cases reported by Evans et al. [1994] and they confirm the occurrence of duodenal atresia and complex congenital cardiac defects in the spectrum of malformations seen in this condition.

Evidence of a link between VACTERL-H and FA was first demonstrated by Porteous et al. [1992]. It had been noted that the spectrum of malformations in severely affected cases of FA could overlap with those seen in the VACTERL association, but Porteous et al. [1992] showed increased mitomycin C (MMC)-induced chromosomal breakage, consistent with a diagnosis of FA, in two unrelated males with VACTERL-H. Subsequently, two further families were reported with VACTERL-H and raised sensitivity to MMC-induced chromosome breakage [Wang et al., 1993; Evans et al., 1994]. In the family described here, there was no evidence of increased spontaneous chromosomal breakage on routine cytogenetic testing. Although testing for increased DEB/MMC sensitivity was not performed, it was possible to demonstrate by molecular analysis that both fetuses were homozygous for the IVS4+4 A→T mutation in *FAC* which is responsible for severe FA in Ashkenazi Jewish individuals. The absence of increased spontaneous chromosomal breakage reinforces the message that if FA is suspected it is essential to test for increased MMC/DEB sensitivity before ruling out

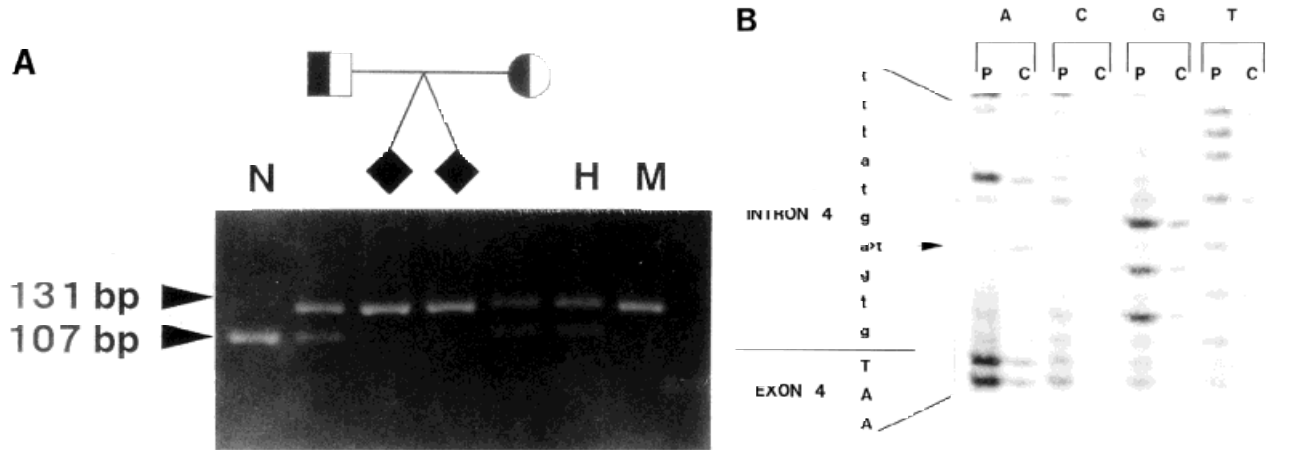

Fig. 3. **A:** Gel photograph of the products of PCR amplification across the IVS4+4 A→T region of *FAC* following *Sca1* digestion. Both parents are heterozygous for IVS4+4 A→T and the wild type allele and generate one normal 108-bp band and one mutant 131-bp band. The twins are both homozygous for IVS4+4 A→T and thus generate a single band of 131-bp. N, normal individual, homozygous for wild type allele; H, heterozygous control with one wild type and one IVS4 4 A T allele; M, homozygous IVS4 +4 A→T control. Size of products in base pairs as indicated; 23-bp band not shown. **B:** DNA sequence analysis of the exon 4/intron 4 boundary. C, control; P, patient (twin 1). Twin 1 is homozygous for the A→T mutation.

the diagnosis. In this regard it is worth noting that a case of VACTERL-H reported by Corsello and Giuffrè [1994], which did not show excess spontaneous chromosome breakage, could still be due to FA. However, a further baby with VACTERL-H, which showed no increase in MMC sensitivity, may well represent a separate syndrome [Evans and Chodirker, 1993]. That case was not typical clinically since its hydrocephalus was the result of an Arnold-Chiari malformation rather than aqueduct stenosis which is usual in VACTERL-H. No clear distinction can be identified between the spectrum of anomalies seen in AR and XLR families with VACTERL-H, and increased chromosome breakage is reported in both groups, raising the possibility of an FA-related gene on the X chromosome.

Cellular complementation studies with FA cell lines indicate that at least 4 genes may independently cause FA [Strathdee et al., 1992a]. By screening for functional complementation of the defect, the gene responsible for complementation group C (*FAC*) was isolated [Strathdee et al., 1992b]. *FAC* is estimated to account for 10–23.5% of FA patients and a variety of *FAC* gene mutations have been identified [Verlander et al., 1994; Whitney et al., 1993; Gibson et al., 1993] which show differences in disease severity [Verlander et al., 1994]. A specific splice site mutation, IVS4+4 A→T in *FAC* appears confined to patients of Ashkenazi Jewish ancestry and is responsible for some 80% of FA in this racial group [Verlander et al., 1994; Whitney et al., 1993, 1994]. Patients with this mutation have a very severe phenotype, with multiple congenital malformations and early onset of bone marrow failure [Verlander et al., 1994].

Until the other FA genes are isolated it will be impossible to perform a full molecular analysis of cases of VACTERL-H, to determine whether or not all cases of VACTERL-H are the result of severe FA gene mutations. Undoubtedly, in the meantime, clinicians and pathologists should be aware of the association between VACTERL-H and FA, so that when a case is encountered the appropriate investigations, i.e., MMC/DEB sensitivity, are undertaken.

ACKNOWLEDGMENTS

The authors thank the family for agreeing to presentation of this case, Dr. C. Mathew for advice during preparation of this paper, Dr. C. Hall for reviewing the X-rays, Mr. J. Bhundia, Mr. P. Temple and Mr. W. Thompson-Ambrose for technical assistance, and Miss M. Lennox-Martin for help preparing the manuscript. R.A.G. and N.M. are supported by the Medical Research Council, U.K.

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